new ligands. Newer approaches aim to dissect the requirements for each step of antigen processing—that is, proteasome cleavage specificity, TAP specificity, and MHC binding specificity (reviewed in Stevanovic [2005]). Such approaches should now consider ERAAP activity on MHC-bound peptides when determining criteria for epitope generation.

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Immunity *25*, November 2006 ©2006 Elsevier Inc. DOI 10.1016/j.immuni.2006.10.007

Human Tyk2 Kinase Deficiency: Another Primary Immunodeficiency Syndrome

Janus kinases are critical for cytokine signaling. Mutations of Jak3 cause primary immunodeficiency, but Minegishi et al. (2006) now show that mutation of another Jak, tyrosine kinase 2, underlies another human immunodeficiency syndrome.

Cytokines regulate immune-cell development, homeostasis, differentiation, and effector function; as such, they have critical roles in host defense and in the pathogenesis of autoimmune and autoinflammatory disorders. A large subset of cytokine receptors (type I and II cytokine receptors) lack intrinsic kinase activity, but instead rely on a small family of receptor-associated cytoplasmic tyrosine kinases to initiate signaling. This family, known as *Ja*nus *k*inases or Jaks, consists of only four members, Jaks1–3 and tyrosine kinase 2 (Tyk2). The essential function of Jaks in effecting cytokine signals has been substantiated by various approaches and is underscored by the fact that Jak3 mutations result in severe combined immunodeficiency (SCID) in humans. Other Jaks were not previously associated with primary immunodeficiency in humans, but Tyk2 can now be added to the list. Minegishi et al. (2006) have identified a patient with Tyk2 deficiency who exhibited broader and more profound immunological defects than would be anticipated from studies of *Tyk2*$^{-/-}$ mice. The signaling defects resulted in a complex clinical picture that included Hyper IgE Syndrome (HIES) and susceptibility to multiple infectious pathogens.

Jaks constitutively associate with the cytoplasmic domains of type I and II cytokine receptors and, upon ligand binding, phosphorylate receptor subunits. This serves to recruit signal transducers and activators of transcription (Stats) and other adapters and signaling molecules. Jaks also phosphorylate and activate Stats, promoting nuclear translocation and transcription. The connection between Jaks and cytokine signaling was first revealed when a screen for genes involved in type I interferon (IFN) signaling identified Tyk2 as an essential element (Velazquez et al., 1992). Shortly thereafter, through the use of somatic mutant cell lines, the first defined connections between different cytokine receptors and different Jaks and Stats were made (Darnell et al., 1994). In vitro stimulations also paired different Jaks with apparent activating cytokines.

The first in vivo proof that Jaks are critical for cytokine signaling came from studies of a group of human disorders termed SCID. Jak3 selectively associates with the common γ cytokine receptor chain (γc), and mutations in γc were known to cause SCID (X-SCID). Mutations of *JAK3* were therefore sought and found to underlie some cases of autosomal recessive SCID (Macchi et al., 1995); a similar phenotype was also observed in Jak3-deficient mice. Several years later, Jak1- and Jak2-deficient mice were produced, but it was not until 2000 that Tyk2-deficient mice were generated (Karaghiosoff et al., 2000; Shimoda et al., 2000). In contrast to Jak3, which appears to associate with only one receptor subunit, Tyk2 is activated by an array of cytokine receptors. It was therefore striking that Tyk2 deficiency in mice identified somewhat limited roles for this kinase, primarily in interleukin 12 (IL-12) and interferon (IFN-) α and β signaling.

In a compelling new study, Minegishi et al. (2006) identified a patient homozygous for a *TYK2* mutation

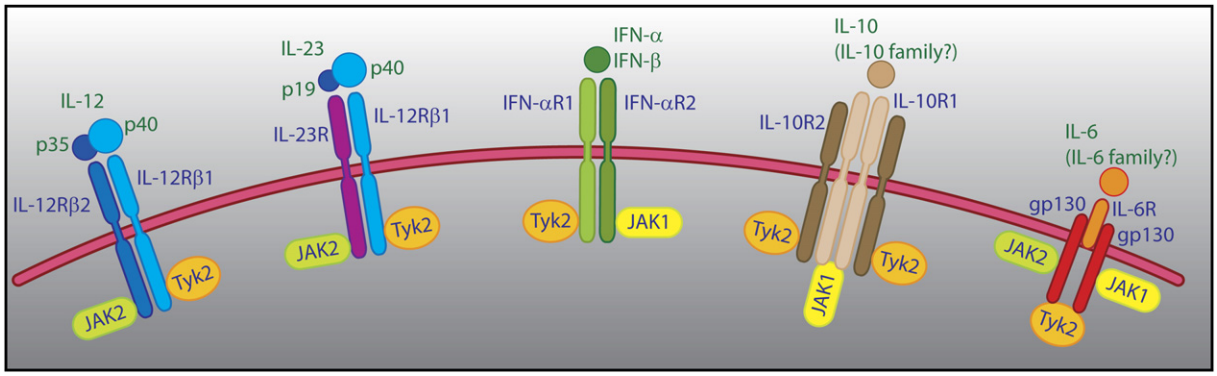

Figure 1. Cytokines and Receptors that Utilize Tyk2

Minegishi et al. (2006) show that Tyk2 has broader and more profound functions in humans than previously appreciated on the basis of analysis of murine models. Murine models indicate that Tyk2 functions primarily in IL-12 and type I IFN signaling. However, the current study shows that in addition to IFN-α and -β and IL-12 signaling, Tyk2 has major effects on the transduction of IL-23, IL-10, and IL-6 signals. Because IL-6 signals through the gp130 receptor chain that is common to a large family of cytokines, including IL-6, IL-11, IL-27, IL-31, oncostatin M (OSM), ciliary neurotrophic factor, cardiotrophin-1, cardiotrophin-like cytokine, and leukemia inhibitory factor (LIF), Tyk2 might also affect signaling through these cytokines. Similarly, IL-22 is a member of the IL-10 family thought to signal through Tyk2, but its contribution to the patient's disease is unknown.

that resulted in a premature termination codon and absence of Tyk2 protein. The patient exhibited diverse symptoms, demonstrating the nonredundant functions of this Jak in signaling via several receptors (Figure 1). Comparisons of cytokine responsiveness in immune cells from the human Tyk2-deficient patient and Tyk2-deficient mice are in some respects predictable. In general, though, the authors show that Tyk2 deficiency has more profound consequences in human cells than would be expected from studies in murine cells.

First, the patient had increased susceptibility to viral infections, pointing to the criticality of Tyk2 in transmitting signals from the IFN-α and -β receptor (IFNAR). This is consistent with the documented role of Tyk2 in mediating IFN signals; however, Tyk2 deficiency has more dramatic effects in human cells than in mouse cells. IFN-α-induced phosphorylation of other relevant signaling molecules, such as Jak1 and Stat1, was completely abrogated in the human Tyk2-deficient cells, whereas this treatment induced substantial residual phosphorylation of these proteins in the Tyk2-deficient mice (Karaghiosoff et al., 2000; Shimoda et al., 2000). Additionally, original studies using mutant human cell lines argued for a role of Tyk2 in the proper trafficking of IFNAR1 to the plasma membrane. Consistent with this observation, human Tyk2 deficiency leads to decreased surface expression of IFNAR1—this, too, is different from the mouse, where there are normal levels of IFNAR1 and 2 surface expression despite Tyk2 deficiency (Karaghiosoff et al., 2000; Shimoda et al., 2000).

The patient also suffered from atypical mycobacterial infections, and deficits in the IL-12 and IFN-γ axis are known to result in susceptibility to these pathogens (Reichenbach et al., 2001). The authors show that Tyk2 is critical for IL-12 signaling in humans. Lack of IL-12 signaling resulted in impaired T helper 1 differentiation and IFN-γ production. IFN-γ treatment is effective for some patients, but whether Tyk2-deficient patients would be responsive is uncertain because absence of this kinase also evidently impairs IFN-γ signaling. This appears to be due to a reduction in total Stat1 levels rather than impaired proximal IFN-γ signaling, which relies on Jak1 and Jak2. Thus, impairment in IFN-γ signaling is another contributor to impaired host defense, which may have important consequences with respect to therapy in these patients.

Another feature of this patient's disease was atopic dermatitis, elevated levels of immunoglobulin (Ig) E, and exaggerated in vitro T helper (Th)2 differentiation with increased production of IL-5 and IL-13. The clinical manifestations are consistent with defective IL-12 signaling in that impaired Th1 differentiation with skewing toward Th2 polarization would be expected in this setting. More recently, though, it has been recognized that IL-12 and IL-23 share ligand and receptor subunits, and both activate Tyk2. IL-23 is thought to be important in maintenance of IL-17-producing cells. IL-23 and IL-17 are important in the pathogenesis of autoimmune disease and host defense against extracellular bacteria. Previous studies in mice pointed to a key role of Tyk2 in IL-23 signaling (Shaw et al., 2003). Interestingly, the Tyk2-deficient patient also suffered from skin abscesses. It is possible that one aspect of the pathogenesis of this immunodeficiency is the failure to produce IL-17. This was not explored by the authors but is clearly an area worthy of future study. IL-23 also induces the production of IL-22, which in turn induces the production of defensins in skin; this too may contribute to the patient's disease.

IL-10 is a critical anti-inflammatory cytokine, and *Il10*$^{-/-}$ mice suffer from fatal, systemic autoimmune disease. Tyk2 is also activated by IL-10, and Tyk2 deficiency affects the ability to generate and respond to IL-10 (Shaw et al., 2006). Studies of the Tyk2 patient cells also showed defective IL-10 signaling. Why then did the patient not also have autoimmune disease? It is tempting to speculate that impaired IL-12 and IL-23 signaling might attenuate manifestations of autoimmunity; clearly, this aspect of the patient's disease is worthy of further consideration. Additionally, a number

of other cytokines are in the IL-10 family (IL-19, IL-20, IL-22, and IL-26), and the extent to which they depend upon Tyk2 was not evaluated.

A difference noted in the present study versus what was seen in cells from Tyk2-deficient mice is responsiveness to IL-6. IL-6 signaling in the mouse appears to be Tyk2-independent, whereas IL-6 signaling in the patient cells was partially Tyk2 dependent. This is of interest because IL-6 utilizes the gp130 receptor chain, which is common to a large family of cytokines including IL-6, IL-11, IL-27, IL-31, oncostatin M (OSM), ciliary neurotrophic factor, cardiotrophin-1, cardiotrophin-like cytokine, and leukemia inhibitory factor (LIF). More studies will be needed to determine which, if any, other gp130 cytokine members are affected by Tyk2 deficiency and whether these deficits are clinically relevant.

In conclusion, the "experiment of nature" described herein clearly establishes the critical role of Tyk2 in humans, and the signaling defects found correlate well with the clinical presentation. How common is this disorder? Apparently, not very common; in fact, the authors carefully point out that this genetic lesion may be the determinant for only a subset of patients with recessive HIES. Notably, the autosomal-dominant form of HIES is associated with skeletal abnormalities, and it is clear that this is a distinct clinical entity. However, the findings are also of potential interest with respect to the development of immunosuppressive drugs. Because Jak3 deficiency is associated with profound immunodeficiency, considerable effort has been exerted in developing a clinically useful Jak3 antagonist (Changelian et al., 2003). Such a drug has been developed and is currently being studied in clinical trials in the setting of transplant rejection, rheumatoid arthritis, and psoriasis. Given that the development of a selective Jak antagonist is feasible and given the emerging information on the IL-23 and IL-17 axes in immune-mediated disease, development of a Tyk2 inhibitor appears to be a reasonable strategy. Like all other immunosuppressive drugs, a Tyk2 inhibitor would not be without risks. Analysis of Tyk2-deficient patients can give clues as to what to expect in humans treated with a Tyk2 antagonist.

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Immunity *25*, November 2006 ©2006 Elsevier Inc. DOI 10.1016/j.immuni.2006.10.005

Thymus Exclusivity: All the Right Conditions for T Cells

A fraction of primitive "lymphoid" precursors retain plasticity for myeloid differentiation. In this issue of *Immunity*, Laiosa et al. describe that Notch-Delta signals can protect thymic precursors from reprogramming into the myeloid lineage, antagonizing the enforced myeloid transcription factors such as PU.1 and C/EBPα.

For efficient T cell production, primitive hematopoietic progenitors require the influence of the thymic microenvironment. However, the progenitor population that directly seeds the thymus is still controversial (Traver and Akashi, 2004). In the bone marrow, at least three independent "lymphoid" progenitor subsets are responsible for seeding the thymus to develop T cells. These include IL-7Rα$^+$Sca-1^{lo}c-Kit^{lo} common lymphoid progenitors (CLPs), IL-7Rα$^+$pTα$^+$B220$^+$CD19$^-$c-Kit$^-$ cells (CLP-2), and IL-7Rα$^-$RAG1$^+$Sca-1$^+$c-Kit$^+$ earliest lymphocyte progenitors (ELPs), all of which possess robust T cell potential in vivo (Figure 1; Traver and Akashi, 2004). Although CLPs do not generate granulocyte-monocyte (GM) lineage cells, RAG1-expressing ELPs still possess a minor GM potential. Immediately after intravenous transplantation of whole bone marrow cells into irradiated hosts, a substantial fraction of thymic immigrants contains progenitors with GM potential (Mori et al., 2001). In the steady-state thymus, the CD25$^-$CD44$^+$c-Kit$^+$ early thymic progenitor subset that should contain